Influence of source and level of glycerin in the diet on growth performance, liver characteristics, and nutrient digestibility in broilers from hatching to 21 days of age

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ABSTRACT The influence of source and level of inclusion of raw glycerin (GLYC) in the diet on growth performance, digestive traits, total tract apparent retention (TTAR), and apparent ileal digestibility of nutrients was studied in broilers from 1 to 21 d of age. There was a control diet based on corn and soybean meal and 8 additional diets that formed a 2 × 4 factorial with 2 sources of GLYC and 4 levels of inclusion (2.5, 5.0, 7.5, and 10%). The GLYC used were obtained from the same original batch of soy oil that was dried under different processing conditions and contained 87.5 or 81.6% glycerol, respectively. Type of processing of the GLYC did not affect any of the variables studied except DM and organic matter retention ($P < 0.05$) that was higher for the 87.5% glycerol diet. From d 1 to 21, feed conversion ratio (FCR) improved linearly (L, $P \leq 0.01$) as the GLYC content of the diet increased, but ADG was not affected. On d 21, the relative weight (% BW) of the liver and the digestive tract increased (L, $P < 0.01$) as the level of GLYC in the diet increased, but lipid concentration in the liver was not affected. The TTAR of DM and organic matter increased quadratically (Q, $P < 0.05$) and the AME_n content of the diet increased linearly (L, $P < 0.01$) with increases in dietary GLYC. Also, the apparent ileal digestibility of DM (L, $P < 0.05$; Q, $P = 0.07$) and gross energy (L, $P < 0.01$) increased as the GLYC content of the diet increased. It is concluded that raw GLYC from the biodiesel industry can be used efficiently, up to 10% of the diet, as a source of energy for broilers from 1 to 21 d of age and that the energy content of well-processed raw GLYC depends primarily on its glycerol content.

Key words: apparent metabolizable energy, broiler performance, glycerol, ileal digestibility, liver fat

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INTRODUCTION

Raw glycerin (**GLYC**), a byproduct of the biodiesel industry, is an attractive energy source that can replace part of the of cereal grain in poultry diets (Swiatkiewicz and Koreleski, 2009). Raw GLYC contains variable amounts of water, glycerol, and ash and is generally recognized as safe when used in accordance with good manufacturing and feeding practices (Code of Federal Regulations, 2004; EFSA, 2010). The AME_n content of GLYC in poultry ranges from 3,254 to 4,134 kcal/kg (Lammers et al., 2008; Dozier et al., 2011; McLea et al., 2011), values that corresponded to approximately 96% of the gross energy (**GE**) supplied by the glycerol contained in the commercial GLYC. The maximum level of dietary GLYC for optimal growth performance of broilers has been estimated within the range of 5 to 10% (Simon et al., 1996; Cerrate et al., 2006; Dozier et al., 2008).

Glycerol is an important structural component of triglycerides and phospholipids that is easily absorbed in the gastrointestinal tract (**GIT**) of poultry and mammals. Glycerol is a precursor of glyceraldehyde 3-phosphate, an intermediate in the lipogenesis and gluconeogenesis pathways, and can yield energy through the glycolytic and tricarboxylic acid pathways (Lin, 1977; Brisson et al., 2001; Lammers et al., 2008). Kerr et al. (2011) indicated that glycerol metabolism largely occurs in the liver of nonruminant animals. Cryer and Bartley (1973) reported that when glycerol is included at high levels in the diet, it induces anatomical, physiological, and biochemical changes in the liver of rats. However, Lin et al. (1976) observed no effects of dietary glycerol on fatty acid synthesis in the liver or relative weight of the liver in chickens. To our knowledge, no information is available regarding the effect of GLYC inclusion in the diet on apparent ileal digestibility (**AID**)

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of nutrients and liver variables in modern strains of broilers. The aim of this research was to evaluate the effect of source and level of raw GLYC in the diet on growth performance, liver variables, and nutrient digestibility in broilers from 1 to 21 d of age.

MATERIALS AND METHODS

All experimental procedures were approved by the animal Ethics Committee of the Universidad Politécnica de Madrid and were in compliance with the Spanish Guidelines for the Care and Use of Animals in Research (Boletín Oficial del Estado, 2007).

Husbandry

In total, 630 one-day-old Ross 308 chicks were obtained from a commercial hatchery and distributed at random with an equal number of males and females into 63 cages (1.0 m × 0.9 m; Alternative Design, Siloam Springs, AR). The cages were provided with wire flooring and equipped with 2 nipple drinkers and an open trough feeder. Room temperature was kept at 33°C during the first 3 d of life and then it was reduced gradually according to age until reaching 24°C at 21 d. Chicks received a 23L:1D light program for the first 7 d of life and then 20L:4D until the end of the experiment. Broilers had free access to feed and water throughout the experiment.

Raw GLYC, Diets, and Experimental Design

The GLYC sources used were provided by Bio-Oil Huelva, S. L. (Huelva, Spain) and resulted from the process of biodiesel production from soybean oil. A batch of GLYC was divided into 2 portions and heated at either 146 ± 2°C or 151 ± 2°C for 30 min. Consequently, the main difference between the 2 sources of GLYC was the amount of water that remained in the commercial product after the process (4.8 vs. 10.7%) and the glycerol content (87.5 vs. 81.6%). The estimated AME_n concentrations of the 2 GLYC sources were 3,631 and 3,474 kcal/kg, respectively, values that corresponded approximately to 96% of the GE of glycerol. There were 9 experimental diets that consisted of a corn and soybean meal control diet without any GLYC inclusion and 8 additional diets organized as a 2 × 4 factorial with 2 sources of GLYC (**H-GLYC** with 87.5% glycerol and **L-GLYC** with 81.6% glycerol) at 4 levels of inclusion (2.5, 5.0, 7.5, and 10.0%). For the manufacturing of the feeds, the control diet and the 2 diets that contained 10% GLYC were formulated (Fundación Española Desarrollo Nutrición Animal, 2010). Within each of the 2 GLYC set of diets, the intermediate feeds were obtained by judicious mixing in adequate proportions of the summit diets. Each treatment was replicated 7 times, and the experimental unit was a cage with 10 birds. All diets were presented in crumble form and had similar nutritive value (3,000 kcal of AME_n/kg, 21.8% CP, and 1.20% digestible Lys) according to Fundación Española Desarrollo Nutrición Animal (2008). As the level of GLYC in the diet increased, the amount of corn and soybean oil decreased and that of soybean meal increased. Celite, an acid-washed diatomaceous earth (Celite Hispánica S.A., Alicante, Spain), was added at 1% to all diets to increase the acid insoluble ash content of feeds, ileal digesta, and excreta. The chemical composition of the GLYC sources and the ingredient composition and nutritive value of the experimental diets are shown in Tables 1 and 2, respectively.

Table 1. Chemical analyses of the glycerin sources (%, as-fed basis, unless otherwise stated)

Item	L-GLYC[1]	H-GLYC[2]
DM	89.3	95.2
Gross energy (kcal/kg)	3,578	3,820
Glycerol	81.6	87.5
Ash	5.56	5.59
Ether extract	0.25	0.32
Nitrogen	0.13	0.17
Sodium chloride	4.4	3.9
Methanol	0.06	0.05
Nonglyceride organic matter[3]	2.1	1.3
AME_n[4] (kcal/kg)	3,474	3,631
pH	5.3	5.3

[1]Raw glycerin containing 81.6% glycerol.

[2]Raw glycerin containing 87.5% glycerol.

[3]Defined as the difference between 100 and the percentage of glycerol, water, and ash of the raw glycerin (Hansen et al., 2009).

[4]Calculated assuming that the AME_n for glycerol was 0.96 × gross energy.

Growth Performance and Digestive Trait Measurements

Body weight and feed consumption were determined by cage at 7, 14, and 21 d of age. Feed wastage was recorded daily in pans placed beneath the cages, and mortality was recorded and weighed as produced. From these data, ADG, ADFI, and feed conversion ratio (**FCR**) were determined by week and for the entire experimental period.

At 21 d of age, 2 birds per cage replicate were randomly selected, weighed individually, and slaughtered by CO_2 inhalation. The GIT, from the beginning of the proventriculus to the cloaca, including digesta content, liver, spleen, and pancreas, was removed aseptically and weighed. Then, the liver was excised and weighed. The weight of the full GIT and liver was expressed as a percentage of BW. The livers were stored in a freezer at −20°C, and representative samples were freeze-dried, ground using a mortar and pestle to pass through a 0.5-mm screen, and mixed by replicate. Samples were maintained in airtight containers at 4°C until chemical analyses.

Table 2. Ingredient and chemical composition of the experimental diets (%, as-fed basis, unless otherwise stated)

Item	Control	L-GLYC[1]				H-GLYC[2]			
		2.5	5.0	7.5	10.0	2.5	5.0	7.5	10.0
Ingredient									
Corn	56.14	53.36	50.59	47.81	45.03	53.38	50.63	47.87	45.11
Soybean meal (47.5% CP)	34.70	35.15	35.60	36.05	36.50	35.15	35.60	36.05	36.50
Soybean oil	3.50	3.43	3.35	3.28	3.20	3.38	3.25	3.13	3.00
Raw glycerin	—	2.50	5.00	7.50	10.00	2.50	5.00	7.50	10.00
Calcium carbonate	0.70	0.70	0.70	0.69	0.69	0.73	0.75	0.78	0.80
Dicalcium phosphate	2.34	2.35	2.35	2.36	2.36	2.35	2.35	2.36	2.36
Sodium chloride	0.46	0.37	0.28	0.19	0.10	0.37	0.28	0.19	0.10
DL-Met (99%)	0.31	0.31	0.32	0.32	0.32	0.31	0.32	0.32	0.32
L-Lys-HCl (78.5%)	0.27	0.26	0.25	0.24	0.23	0.26	0.26	0.25	0.24
L-Thr (99%)	0.08	0.08	0.08	0.07	0.07	0.08	0.08	0.07	0.07
Vitamin and mineral premix[3]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Celite[4]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Calculated analysis[5]									
AME_n (kcal/kg)	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000
Calcium	0.95	0.95	0.95	0.96	0.97	0.95	0.95	0.96	0.97
Available phosphorus	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46
Digestible Lys	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Digestible Met	0.59	0.59	0.59	0.60	0.60	0.59	0.59	0.60	0.60
Digestible Thr	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
Digestible Trp	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Digestible Arg	1.28	1.29	1.29	1.30	1.30	1.29	1.29	1.30	1.30
Determined analysis[6]									
Gross energy (kcal/kg)	4,051	4,048	4,082	4,102	4,100	4,056	4,093	4,098	4,104
DM	89.1	89.7	89.6	90.4	89.2	90.6	91.0	92.0	89.9
CP	21.2	21.0	20.9	21.3	21.0	20.8	21.1	21.3	21.0
Total ash	6.3	6.6	6.6	6.7	6.9	6.8	6.7	6.9	7.1
Ether extract	7.2	7.1	6.9	7.0	6.9	6.9	7.0	6.9	6.8

[1]Raw glycerin containing 81.6% glycerol.

[2]Raw glycerin containing 87.5% glycerol.

[3]Provided the following (per kilogram of diet): vitamin A (transretinyl acetate), 10,000 IU; vitamin D_3 (cholecalciferol), 2,000 IU; vitamin E (all-*rac*-tocopherol acetate), 20 IU; vitamin K (bisulfate menadione complex), 3 mg; riboflavin, 5 mg; pantothenic acid (D-calcium pantothenate), 10 mg; nicotinic acid, 30 mg; pyridoxine (pyridoxine·HCl), 3 mg; thiamine (thiamine-mononitrate), 1 mg; vitamin B_{12} (cyanocobalamine), 12 μg; D-biotin, 0.15 mg; choline (choline chloride), 300 mg; folic acid, 0.5 mg; Se (Na_2SeO_3), 0.1 mg; I (KI), 2.0 mg; Cu ($CuSO_4{\cdot}5H_2O$), 10 mg; Fe ($FeSO_4{\cdot}7H_2O$), 30 mg; Zn (ZnO), 100 mg; Mn ($MnSO_4{\cdot}H_2O$), 100 mg; and ethoxyquin, 110 mg.

[4]Acid-washed diatomaceous earth (Celite Hispánica, S.A., Alicante, Spain).

[5]According to Fundación Española Desarrollo Nutrición Animal (2010).

[6]In triplicate.

Nutrient Retention and Apparent Ileal Digestibility

At 21 d of age, representative samples of the excreta produced during the previous 48 h were collected daily by replicate, homogenized, oven-dried (60°C for 72 h), ground using a hammer mill (model Z-I, Retsch, Stuttgart, Germany) fitted with a 1-mm screen, and mixed. The total tract apparent retention (**TTAR**) of DM, organic matter (**OM**), GE, and nitrogen (**N**) of the diets was estimated by the indigestible marker method using celite as inert marker. The AME_n content of the diets, adjusted for N retention, was also determined as indicated by Lázaro et al. (2003). After the determination of the TTAR of the nutrients, all the remaining birds (9–10 broilers per cage) were slaughtered by asphyxiation in CO_2 atmosphere, and the ileal section of the small intestine from the vitelline Meckel's diverticulum to 2 cm anterior to the ileocecal junction was immediately removed and the digesta collected by gently flushing the contents with distilled water into plastic containers. Samples were pooled, homogenized, frozen at −20°C, and freeze-dried. Then, the dried samples were ground using a mortar and pestle to pass through a 0.5-mm screen and maintained in airtight containers at room temperature until determination of the AID of DM, N, and GE.

Laboratory Analyses

Samples of GLYC, diets, and excreta were analyzed for moisture (oven-drying, method 930.01), total ash (muffle furnace, method 942.05), and N (combustion method, 990.03) using a Leco model FP-528 (Leco Corporation, St. Joseph, MI) as described by AOAC International (2000). Gross energy was determined using an adiabatic bomb calorimeter (model 356, Parr Instrument Company, Moline, IL). The ether extract of GLYC and diets was determined after 3 *N* HCl acid hydrolysis (method Am 5–04) as described by AOCS (2004) using an Ankom XT10 Extraction system (Ankom Technology Corp., Macedon, NY). The GLYC sources were analyzed also for glycerol (method Ea 6–51) and sodium chloride (method Ea 2–38) content as described by AOCS (2000). Methanol content was determined according to method EN 14110 of the European Standard (2003) and the pH using a digital pH meter (model 507, Crison Instruments S. A., Barcelona, Spain). The nonglyceride organic matter of the GLYC, defined as the difference between 100 and the percentage of glycerol, water, and ash, was also calculated (Hansen et al., 2009). Ileal samples were analyzed for DM, N, and GE as indicated for the diets. Acid insoluble ash of diets, excreta, and ileal contents were determined as indicated by De Coca-Sinova et al. (2011). The lipid content of the livers was determined as indicated by Folch et al. (1957). In addition, the color of the liver [lightness (L*), redness (a*), and yellowness (b*)] was measured by spectrophotometry (CM-700d/600d, Konica Minolta Company, Osaka, Japan) at a wavelength range from 400 to 700 nm. All analyses were conducted in triplicate for feed samples and livers and in duplicate for GLYC, excreta, and ileal contents.

Statistical Analysis

Data on growth performance, GIT weight, liver characteristics, and TTAR and AID of nutrients were analyzed as a completely randomized design using the GLM procedure of SAS (SAS Institute Inc., 1990). For the 8 diets arranged as a 2 × 4 factorial, the main effects (source and level of GLYC inclusion) and the interaction were studied. In addition, treatment sums of squares for GLYC content of the feeds were partitioned into linear (**L**) and quadratic (**Q**) responses to increasing levels of GLYC in the diet (0, 2.5, 5.0, 7.5, and 10.0%). Results in tables are reported as least squares means. When the model was significant, the Tukey test was used to make pairwise comparisons between treatment means. Differences among treatments were considered significant at $P < 0.05$.

RESULTS

The determined chemical analyses of the GLYC sources were close to planned values (Table 1). As expected, the main differences between the 2 GLYC sources tested were the moisture (9.7 vs. 4.8%) and the glycerol (81.6 vs. 87.5%) contents. The methanol concentration of the 2 samples of GLYC was low (600 and 500 mg/kg, respectively) and was not expected to create any metabolic problem in the chicks (Dozier et al., 2011; Jung and Batal, 2011). The determined nutritive values of the experimental diets were close to expected values, confirming that the diets were mixed correctly (Table 2).

Growth Performance and Digestive Trait Measurements

No interactions between source and level of GLYC in the diet were observed for any of the traits studied and therefore, only main effects are presented (Tables 3 and 4). Type of GLYC did not affect broiler performance or GIT traits in any of the periods considered. However, the inclusion of GLYC in the diet tended to improve ADG (L, $P = 0.108$) and improved FCR (L, $P < 0.01$) from 1 to 21 d of age. Most of the beneficial effects of GLYC inclusion on broiler performance were observed during the first days of life. In this respect, the FCR from d 1 to 7 (L, $P < 0.01$) and from d 7 to 14 (L, $P = 0.\ 095$) were improved as the GLYC content of the diet increased, but no benefits were detected from d 14 to 21. The inclusion of GLYC in the diet increased the relative weight of the GIT (L, $P < 0.01$) and the liver (L, $P = 0.01$), but lipid content and color variables of the liver were not affected (Table 4).

Nutrient Retention and Apparent Ileal Digestibility

No interactions between source and level of dietary GLYC were detected for TTAR or AID of the nutrients, and therefore, only main effects are presented (Tables 5 and 6). The TTAR of DM ($P \leq 0.05$), OM ($P = 0.05$), and GE ($P = 0.092$) were higher for the H-GLYC diets than for the L-GLYC diets. Also, DM (Q, $P \leq 0.05$) and OM (Q, $P \leq 0.05$) retention increased as the level of GLYC in the diet increased with the highest retention values reported with 2.5 to 5.0% GLYC inclusion. Moreover, the AME_n of the diet increased as the level of GLYC increased (L, $P \leq 0.01$). The inclusion of GLYC in the diet increased the AID of DM (L, $P = 0.01$; Q, $P = 0.067$) and GE (L, $P < 0.01$; Q, $P = 0.102$). However, neither TTAR nor AID of N was affected by diet.

DISCUSSION

Growth Performance and Digestive Trait Measurements

In the current experiment, type of GLYC did not affect broiler performance, a finding that was expected because diets were formulated using different AME_n values for the 2 GLYC used. The AME_n content of GLYC in poultry reported in the literature varies widely, but most of the differences observed among researchers are due primarily to the glycerol content of the GLYC batch used. The AME_n content of the H-GLYC (87.5% glycerol) and L-GLYC (81.6% glycerol) used in the current experiment was 3,631 and 3,474 kcal/kg, respectively, values that compare well with the value of 3,540 kcal/kg recommended by Fundación Española Desarrollo Nutrición Animal (2010) for a commercial GLYC with 85% glycerol. In fact, the energy content (per unit of glycerol) used in the current research for the GLYC was slightly lower than the value of 3,621 kcal/kg determined by Dozier et al. (2008) and of 3,487 kcal/kg

Table 3. Influence of source and level of raw glycerin (GLYC) in the diet on growth performance of broilers

	1–7 d			7–14 d			14–21 d			1–21 d		
Item	ADG (g)	ADFI (g)	FCR[1]	ADG (g)	ADFI (g)	FCR	ADG (g)	ADFI (g)	FCR	ADG (g)	ADFI (g)	FCR
GLYC source (%)												
L-GLYC[2]	21.5	23.2	1.08	42.0	53.4	1.27	47.6	72.9	1.53	37.0	49.2	1.33
H-GLYC[3]	21.7	23.4	1.08	41.9	53.2	1.27	47.7	72.5	1.52	37.1	49.2	1.33
GLYC level (%)												
0.0	21.7	23.8	1.10	41.3	53.3	1.29	46.2	71.5	1.55	36.4	49.2	1.35
2.5	21.7	23.4	1.08	42.3	53.8	1.28	47.1	71.9	1.53	37.0	49.3	1.33
5.0	21.8	23.5	1.08	41.9	52.4	1.25	47.9	72.3	1.51	37.2	49.0	1.32
7.5	22.0	23.6	1.08	42.6	54.3	1.28	47.7	72.9	1.53	37.4	49.7	1.33
10.0	21.0	22.4	1.07	41.7	52.8	1.27	49.6	75.0	1.52	37.4	49.0	1.31
SEM[4]	0.45	0.43	0.01	0.89	0.93	0.01	1.71	2.27	0.02	0.65	0.79	0.01
P-value[5]												
GLYC source	0.445	0.613	0.660	0.824	0.692	0.777	0.966	0.829	0.600	0.917	0.959	0.637
GLYC level												
Linear	0.213	0.008	0.006	0.601	0.779	0.095	0.057	0.121	0.208	0.108	0.967	0.002
Quadratic	0.160	0.230	0.466	0.225	0.790	0.075	0.841	0.528	0.391	0.476	0.764	0.453

[1]Feed conversion ratio.

[2]Raw GLYC containing 81.6% glycerol.

[3]Raw GLYC containing 87.5% glycerol.

[4]Standard error of the mean (n = 7 replicates of 10 birds each).

[5]The interaction between source and level of GLYC was not significant ($P > 0.10$).

determined by McLea et al. (2011) for GLYC samples containing 86.9 and 81.0% glycerol, respectively.

The inclusion of up to 10% GLYC in the diet improved growth performance of the broilers with effects being more pronounced for FCR than for ADG and for the first week of life than thereafter, consistent with data of Lima et al. (2012), who reported that young broilers have a higher capacity to use the energy content from this ingredient than older broilers. McLea et al. (2011) and Topal and Ozdogan (2013) reported also that the inclusion of 8 to 10% glycerol in the diet improved ADG and FCR in broilers from 1 to 28 d of age, in agreement with the results of the current experiment. Similarly, Yalçın et al. (2010) observed that the inclusion of 7.5% GLYC in the diet improved FCR in laying hens from 39 to 55 wk of age. In contrast, Simon et al. (1996) and Cerrate et al. (2006) reported that the inclusion of 5 or 10% GLYC in the diet did not affect FCR of broilers although ADG was also improved.

In the current research, broilers used more efficiently the diets that contained GLYC than the diet containing no GLYC, suggesting that the real contribution of GLYC to the energy content of the diet was higher than calculated based on the estimate of 4,160 kcal of AME_n per kg of glycerol of the GLYC of the Fundación Española Desarrollo Nutrición Animal (2010; e.g., 3,540 kcal of AME_n/kg for the H-GLYC that contained 85% glycerol). The experimental diets were formulated on ME bases rather than on net energy bases. Consequently, the real contribution of glycerol to the energy content of the diet was underestimated because glycerol is absorbed by the bird more rapidly and efficaciously (without any heat loss) than corn (Simon et al., 1997). In addition, because of its physico-chemical properties, GLYC inclusion may affect digesta transit time in the GIT of the birds, improving the utilization of other components of the diet, as has been demonstrated for fats by Mateos and Sell (1980, 1981). The data provided indicate that the coefficient of utilization of the GE of glycerol contained in the GLYC averaged 96% in young broilers, a value that is higher than the range of 85.0 to 90.3% reported for poultry by Alvarenga et al. (2012) and for nursery and growing pigs by Kerr et al. (2009) and Kovács et al. (2011).

The effects of dietary GLYC on the size and fat content of the liver have not been studied extensively in broilers, and in fact, the information available is conflicting. Sehu et al. (2012) observed that the weight of the liver of 42-d-old broilers increased when 10% GLYC was included in the diet, in agreement with the results reported herein. In the liver, glycerol can be converted into glucose and used to yield energy or for synthesis of triglyceride and phospholipid (Robergs and Griffin, 1998). Cryer and Bartley (1973) suggested that the increase in liver size observed in rats fed high levels of GLYC was caused by increased gluconeogenesis that resulted in overloading of liver function. In contrast, Lessard et al. (1993) and Topal and Ozdogan (2013) reported no effects on liver size in broilers of similar age

Table 4. Influence of source and level of raw glycerin (GLYC) in the diet on gastrointestinal tract (GIT) weight, and weight, lipid, and color of the liver in broilers at 21 d of age

Item	BW (g)	Relative weight (% of BW)		g of lipid/100 g of liver	Liver color[2]		
		GIT[1]	Liver		L*	a*	b*
GLYC source (%)							
L-GLYC[3]	872	15.0	2.77	12.4	30.1	15.1	10.4
H-GLYC[4]	864	14.8	2.74	12.4	30.5	15.6	10.8
GLYC level (%)							
0.0	846	14.5	2.64	12.1	30.2	14.8	10.9
2.5	862	14.4	2.67	12.4	30.3	16.2	10.8
5.0	864	15.0	2.82	12.4	30.8	15.2	10.4
7.5	852	14.8	2.75	12.7	30.3	15.2	10.5
10.0	894	15.5	2.79	12.3	29.9	15.3	10.4
SEM[5]	20.01	0.35	0.01	0.41	1.03	0.69	0.71
P-value[6]							
GLYC source	0.638	0.326	0.601	0.914	0.500	0.247	0.353
GLYC level							
Linear	0.062	0.003	0.015	0.402	0.769	0.820	0.374
Quadratic	0.482	0.511	0.246	0.367	0.466	0.304	0.883

[1]Weight of the full GIT (from end of the crop to cloaca) including digesta contents and liver, spleen, and pancreas.

[2]Lightness (L*), redness (a*), and yellowness (b*).

[3]Raw GLYC containing 81.6% glycerol.

[4]Raw GLYC containing 87.5% glycerol.

[5]Standard error of the mean (n = 7 replicates of 2 birds each).

[6]The interaction between source and level of GLYC was not significant ($P > 0.10$).

when 5 or 8% GLYC was included in the diet. Moreover, Suchy et al. (2011) reported that the weight of the liver of 40-d-old broilers was reduced when 3% GLYC was included in the diet. All this information suggests that the response of the liver to dietary glycerol might depend on the dose, with increases in liver size when high concentrations of GLYC are used.

Dietary GLYC did not affect fat accumulation in the liver, in agreement with data of Lessard et al. (1993) in chickens and Cryer and Bartley (1973) in rats. In nonruminants, GLYC is metabolized preferably to glucose at a rate that will depend on the metabolic state of the animal and the amount of glycerol consumed (Kerr et al., 2011). In this respect, Lin et al. (1976) reported that the inclusion in the diet of 42.1% glycerol reduced lipogenic enzyme activity in the liver of the chicken. Liver color was not affected by the inclusion of GLYC in the diet, suggesting that glycerol did not affect the macroscopic characteristics and function of the liver. In this respect, Suchy et al. (2011) reported also that the inclusion of 3% GLYC in the diet did not affect the color, shape, structure, or consistency of the liver in broil-

Table 5. Influence of source and level of raw glycerin (GLYC) in the diet on total tract apparent retention (%) of nutrients and AME_n of the diet in broilers at 21 d of age

Item	DM	Organic matter	Nitrogen	Gross energy	AME_n (kcal/kg)
GLYC source (%)					
L-GLYC[1]	71.1	75.5	64.2	77.0	2,907
H-GLYC[2]	71.9	76.2	64.5	77.5	2,927
GLYC level (%)					
0.0	70.6	74.9	64.3	76.5	2,880
2.5	72.0	76.3	64.0	77.6	2,900
5.0	71.9	76.2	64.8	77.6	2,930
7.5	71.6	76.0	64.0	77.2	2,927
10.0	71.5	75.8	64.6	77.4	2,948
SEM[3]	0.42	0.39	0.67	0.35	12.88
P-value[4]					
GLYC source	0.037	0.045	0.626	0.092	0.110
GLYC level					
Linear	0.285	0.188	0.803	0.217	0.006
Quadratic	0.034	0.017	0.908	0.116	0.506

[1]Raw GLYC containing 81.6% glycerol.

[2]Raw GLYC containing 87.5% glycerol.

[3]Standard error of the mean (n = 7 replicates of 10 birds each).

[4]The interaction between source and level of GLYC was not significant ($P > 0.10$).

Table 6. Influence of source and level of raw glycerin (GLYC) in the diet on the apparent ileal digestibility (%) of nutrients in broilers at 21 d of age

Item	DM	Nitrogen	Gross energy
GLYC source (%)			
L-GLYC[1]	69.9	77.9	75.5
H-GLYC[2]	70.1	77.8	75.4
GLYC level (%)			
0.0	67.0	76.8	72.8
2.5	69.4	77.2	74.9
5.0	70.9	78.1	76.1
7.5	72.2	79.4	77.2
10.0	70.5	78.0	76.2
SEM[3]	1.68	1.48	1.44
P-value[4]			
GLYC source	0.841	0.906	0.918
GLYC level			
Linear	0.011	0.158	0.006
Quadratic	0.067	0.413	0.102

[1]Raw GLYC containing 81.6% glycerol.

[2]Raw GLYC containing 87.5% glycerol.

[3]Standard error of the mean (n = 7 replicates of 10 birds each).

[4]The interaction between source and level of GLYC was not significant ($P > 0.10$).

ers. Further research on the effects of level of glycerol consumption on the activity of gluconeogenic enzymes in broilers is needed to evaluate how a metabolic overload of glycerol affects liver size and lipid deposition.

Broilers fed 10% GLYC had heavier digestive tracts than broilers fed the control diet. The authors have not found any full report on the effects of GLYC inclusion in the diet on GIT development in broilers to compare with the results reported herein. In this respect, Topal and Ozdogan (2013) reported no effects on proventriculus and gizzard weight in 42-d-old broilers when 8% GLYC was included in the diet.

Nutrient Retention and Apparent Ileal Digestibility

The TTAR of all nutrients, except N that was not affected, tended to be higher in broilers fed the H-GLYC than in broilers fed the L-GLYC diets, although the differences reported were of little practical interest. Similar results were observed with increases in the level of GLYC of the diet. Diets were formulated to have similar calculated AME_n concentration according to Fundación Española Desarrollo Nutrición Animal (2010), assuming in all cases a metabolicity of 96% for the GE of the glycerol contained in the GLYC. Kim et al. (2013) reported also that the TTAR of DM and GE, but not of N, increased when 5% glycerol was included in the diet of 31-d-old broilers. Similarly, Swiatkiewicz and Koreleski (2009) did not detect any significant effect on N retention when up to 6% GLYC was included in the diet of laying hens, consistent with the results of the current experiment. Also, in pigs, Zijlstra et al. (2009) reported that the TTAD of GE increased as the level of dietary GLYC increased from 0 to 8%. Moreover, Madrid et al. (2013) reported that nutrient digestibility of OM, but not of N, increased when 5% GLYC was included in the diet, although the authors did not provide any explanation for the improvement observed. When GLYC is included in the diet, small variations in ingredient and chemical composition occurred, which might have resulted in the small improvements in nutrient digestibility reported by the different authors.

The AME_n concentration of the diet increased by 2.3% (from 2,880 to 2,948 kcal/kg) as the level of GLYC increased from 0 to 10%. The reason for the improvement in energy content of the diet as the level of GLYC increased is not apparent, but the data are consistent with the higher retention reported for OM and GE. Dozier et al. (2008) reported also a an increase of 1.4% in the AME_n content of the diet in 45-d-old broilers when the GLYC level of the diet increased from 0 to 9%. Similarly, McLea et al. (2011) reported a 5.9% increase in the energy content of the diet as the GLYC level increased from 0 to 10% in 28-d-old broilers. Dietary GLYC might improve nutrient utilization of other ingredients of the diet as has been demonstrated with supplemental fat by Mateos et al. (1982). These authors reported that supplemental fat delays the intestinal transit time of the digesta, allowing a better contact between the components of the digesta and the endogenous enzymes present in the GIT, which in turn could improve nutrient utilization. However, Kim et al. (2013) were not able to detect any benefit of glycerol supplementation on the intestinal transit time of the digesta in 31-d-old broilers. Therefore, other mechanisms might be involved. In this respect, some constituents of the GLYC used, others than the glycerol, might account for the differences observed (EFSA, 2010; Alvarenga et al., 2012). For example, Dozier et al. (2011) reported in 17-d-old broilers higher energy content per unit of glycerol of the GLYC originated from soybean oil than of the GLYC originated from tallow or poultry fat.

The AID of GE and DM increased as the level of GLYC in the diet increased, but that of N was not affected, consistent with the TTAR data. In contrast, McLea et al. (2011) did not detect any influence of the level of dietary GLYC (0 to 10%) on the AID of DM. The authors have not found any other published report on the effects of GLYC inclusion on AID of nutrients in poultry. In the current research, the AID of GE increased as the GLYC content of the diet increased, but GE retention was not affected. The GLYC is almost completely absorbed in the small intestine of the bird, and therefore, GE ileal digestibility was expected to be higher for the GLYC-containing diets. On the other hand, the fermentation process that takes place in the large intestine will increase the utilization of the energy of the remaining substrates in all dietary treatments, and consequently the GE retention of all diets will tend to be equalized.

In summary, raw GLYC can be used at levels of at least 10% in substitution of other energy sources in broiler diets without any negative effect on growth performance. An increase in the level of GLYC in the diet increased TTAR and AID of all nutrients, except N, in broilers at 21 d of age. Also, GLYC inclusion increased the relative weight of the liver, but lipid content and liver color were not affected. Type of GLYC did not affect any of the variables studied except DM, OM, and GE retention, which tended to be higher with the H-GLYC diets. In conclusion, the data indicate that broilers might benefit from the inclusion of moderate (up to 10%) amounts of high-quality GLYC in the diet.

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